Molecular Identity and Spatial Topography of Transient A-type Potassium Channels in the Rostral Nucleus of the Solitary Tract

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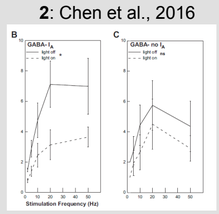
INTRODUCTION

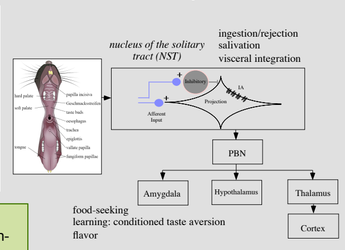
- Overconsumption of food is an undisputed health risk contributing to many diseases & the sense of taste is a major factor influencing food intake.
- The rostral nucleus of the solitary tract (rNST) is the initial central portal through which taste signals pass. This nucleus contains excitatory projection neurons that relay gustatory signals to rostral brain structures that control feeding behavior and GABAergic inhibitory interneurons that modify projection neuron responses (figure 1 below).
- Inhibition is an important function in rNST. For example, gut signals can reduce NST taste responses and may be one mechanism that suppresses feeding after eating (Mattes, 2006).
- Inhibitory processing involves GABAergic inhibitory interneurons and interactions between GABA signals and constitutive membrane channels on projection neurons. One such channel is the outward potassium channel, which produces an A-type K^+ current (Goldwyn et al., 2018).
- Notably, projection neurons with IA are more potently inhibited by GABA neurons than those lacking IA (figure 2 below; Chen et al., 2016).
- However, many fundamental aspects of IA in the rNST are unanswered or controversial including: their (1) molecular identity, (2) precise spatial distribution, and (3) the extent to which these channels also occur in GABAergic neurons (figure 3 below).
- The current study set out to answer these questions using *in vitro* patch-clamp neurophysiology and immunohistochemistry.

2: Chen et al., 2016

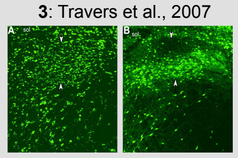


The figure above compares the input-output functions of two non-GABA cells, one with IA and one without IA. A genetically-modified mouse strain allowed the researchers to optogenetically stimulate GABAergic cells, and subsequently inhibit the post-synaptic non-GABAergic cells. Cells with IA demonstrated greater inhibitory properties than cells without IA.

1



3: Travers et al., 2007

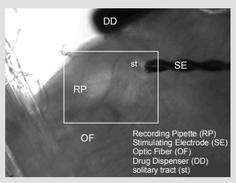


GABA neurons in A were labeled via *in situ* hybridization; B depicts native fluorescence in GABA neurons in the "GIN" mouse, a GAD1-EGFP transgenic mouse used in the 1st *in vitro* study of NST inhibitory neurons. GABA neurons in the GIN mouse are in the ventral subdivision but not the central subdivision whereas *in situ* staining also shows strong expression in the central subdivision. This suggests the possibility that the initial *in vitro* study may have mistakenly concluded that the IA channel is only expressed in non-GABA neurons (Wang and Bradley 2010).

METHODS

Patch-Clamp Setup:
- ❑ In Vitro recording methods, including identifying cell phenotype were performed as in Chen et al., 2016.

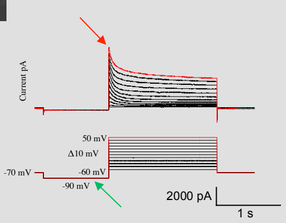
Phenotyping Cells:
- ❑ Cell phenotypes were determined by optogenetic stimulation (1-s trains, 10 Hz, 455-nm light).
- ❑ Cells responses that exhibited inhibitory potentials were classified as non-GABA cells. Neurons with short-latency excitatory potentials were defined as GABAergic



Patch-Clamp Neurophysiology

A brief hyperpolarization of the cell deinactivates the IA channels (Green Arrow)

Subsequent depolarization produces a transient outward current: IA (Red Arrow)

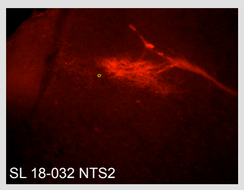


Mice:
- ❑ Strains were chosen that allowed identification of GABAergic and non-GABAergic neurons.
- ❑ The strain used in the *in vitro* studies expressed ChR2-EGFP under the control of the GAD65 promoter. The presence of ChR2 allowed optogenetic identification of neuron phenotype.
- ❑ The main strain used in the immunohistochemical experiments expressed Venus (EGFP) under the control of VGAT (vesicular GABA transporter).

Identification of IA:
- ❑ The presence of IA in a cell was determined by applying a hyperpolarizing voltage (-100 mV, 1 s) followed by incremental increases of depolarizing steps (Max. -40 mV, 5-mV steps, 2 s).
- ❑ *Androctonus mauretanicus mauretanicus* toxin 3 (AmmTX3) was used to block A-type current *in vitro*. The neurotoxin is specific to the Kv4 family of voltage-gated potassium channels (Maffie et al., 2013).

Immunohistochemistry

- ❑ Tissue Processing: Adult mice (4-12 wk) were perfused transcardially with 4% paraformaldehyde, 1.4 % L-Lysine acetate and 0.2% sodium metaperiodiate (~50 mL). After extraction of the brain, the tissue was post-fixed with the solution above and 20% sucrose for 3 hours. The brain stem was blocked, frozen and cut to 40 um coronal sections.
- ❑ The Kv4.3 primary antibody (UC Davis MAB: 74-017) was used to differentially label the IA channels in the rNST. The P2X2 primary antibody (Alomone Labs: APR-003) was used to label the gustatory afferent terminal field. Secondary fluorescent antibodies were subsequently attached to the primary antibodies to demarcate the presence and topography of the IA channels in the rNST (Alexa Fluor 488, 546 and 647).
- ❑ Photomicrographs were taken with either with a Nikon Eclipse microscope equipped with widefield fluorescence (10-20X lenses) or with a confocal microscope (Olympus FV 3000).



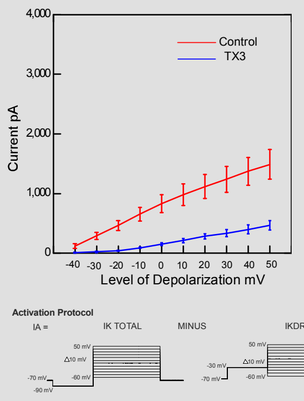
Recording Position Relative to Afferent Termination in the rNST. Photomicrograph corresponds to the rectangle depicted in the Patch-Clamp Set-Up Subheading.

RESULTS

1. Pharmacological Identification of Kv4 Family

1A

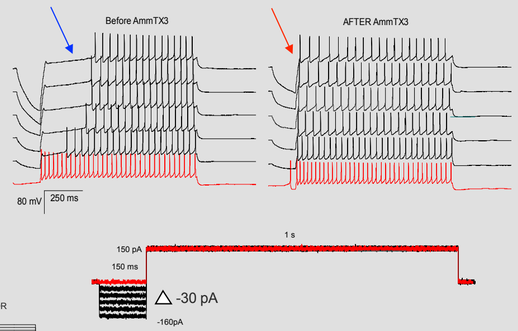
Suppression of IA Current



- Figure 1A compares IA current amplitudes before and after application of AmmTX3. To compute each amplitude value both GABAergic and non-GABAergic cells were held in voltage-clamp and the delayed rectified current (IKDR) was subtracted from the total current (IK). The average amplitude values were determined at each level of depolarization. The neurotoxin significantly reduced the IA current amplitude (n=9; p=0.001).

1B

Elimination of First-Spike Delay

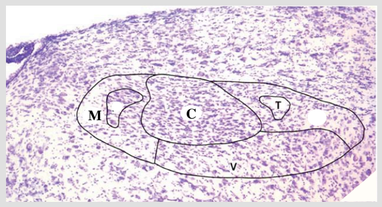


- Figure 1B depicts the current-clamp protocol for measuring IA before and after drug application. The outward IA current causes long first-spike latencies as illustrated by the blue arrow. AmmTX3 drastically reduced the first-spike delay profile as illustrated by the red arrow. The increase in excitability is a direct result from the blocking of outward current IA channels.
- AmmTX3 is specific to the potassium voltage-gated family 4 family (Kv4) (Maffie et al., 2013). Therefore, Figures 1A and 1B suggest that IA in the rNST can be attributed to the Kv4 family.

2. Kv4.3 is one Kv4 family member underlying IA in rNST & is evenly distributed throughout subdivisions

2A

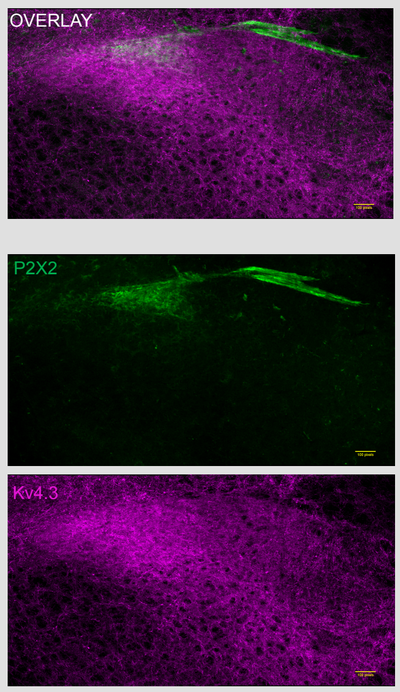
Ganchrow et al. 2013



M, medial C, central L, lateral
V, ventral T, solitary tract

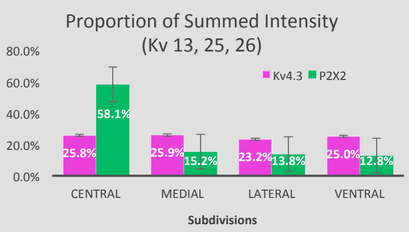
- Figure 2A outlines the subdivisions of the rNST in a Nissl stained mouse model.

2B



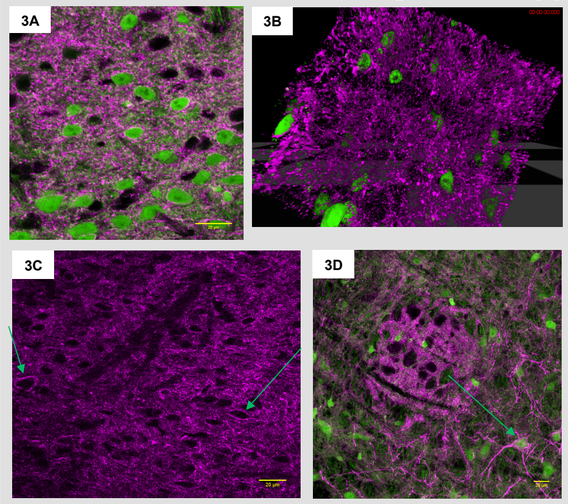
- The photomicrograph in Figure 2B shows the spatial distribution of Kv4.3 labeled channels throughout the rNST.
- The two photos below the overlay split the composite image into P2X2 and Kv4.3 channels. Similar staining patterns were observed in repeated trials (n=26).

2C



- Figure 2C compares the average proportion of summed intensities in each subdivision of the rNST.
- In each subdivision the Kv4.3 proportion is approximately equal.

3. Kv4.3 is strongly expressed in rNST neuropil but some somal labeling is also apparent

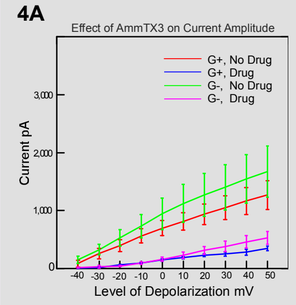


- Confocal images of immunohistochemical staining illustrate the cellular location of Kv4.3.
- Figures 3A and 3B (top) depict immunohistochemical staining for Kv4.3 (magenta) in the rNST in the VGAT-Venus mouse.
- Labeling was frequently located in the neuropil, suggesting a distribution on dendrites or axon terminals.

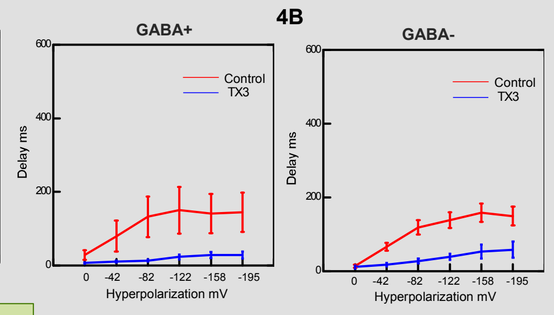
- Figure 3C shows a more atypical observation (from a wild-type mouse): Multiple cells appeared to have somal labeling (arrows).
- Figure 3D shows a GABAergic neuron ventral to NST, just outside the nucleus ambiguus. The cell has both dendritic and soma labeling and serves as a control for rNST neurons.

4. IA is apparent in both non-GABAergic and GABAergic rNST neurons

4A



4B



- The neurotoxin significantly reduced IA in GABAergic and non-GABAergic cells (p=0.001). In contrast to previous findings (Wang and Bradley 2010), this suggests that IA is not exclusive to non-GABAergic cells.

- Figure 4B compares the effectiveness of AmmTX3 in reducing first-spike delay across cell phenotypes. In both cases the drug significantly diminished the delay (p<0.001).
- Additional analysis found a significant difference between hyperpolarization levels and delay values (p<0.001).

Summary & Conclusions

- The outward potassium (IA) current in the rNST is attributed to the potassium voltage-gated family 4 (Kv4). This current was not exclusive to non-GABAergic cells as previously hypothesized (Wang and Bradley 2010), but was present in both GABAergic and non-GABAergic phenotypes.
- Kv4.3 is evenly expressed throughout the rNST.
- Analysis of the spatial location of Kv4.3 channels on individual neurons revealed that Kv4.3 is primarily located in the neuropil, suggesting IA can act in dendrites to modulate inputs to these cells. However, some somal labeling is observed as well.
- The finding that IA appears is mediated by a singular K+ channel family (probably only Kv 4.3) allows on-going experiments in the lab to explore the interaction between IA and inhibition by suppressing IA pharmacologically with a specific channel blocker (AmmTX3) or by adding it to a neuron using the technique of dynamic clamp.
- The presence of IA in a subset of GABAergic neurons suggests the presence of inhibitory influences on these neurons as well, perhaps creating a mechanism for modulation of "disinhibition", a mechanism that has been postulated to play a role in taste processing within the rNST (e.g., Kinzeler & Travers, 2011; Smith & Li, 1998)

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